

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**

**MEMORANDUM OF CONFERENCE**  
**September 20, 1994**

**Participants:**

Industry:

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Robert Sacher	Hunt-Wesson, Inc.
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FDA:

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**Subject:** Processing tomatoes having suppressed polygalacturonase (PG) activity

**Introduction**

This meeting was intended to bring Zeneca's consultation started in the Fall of 1992 to closure (see SBJ 1319). Zeneca had previously submitted (September 6, 1994) a summary of the safety assessment of their transgenic tomato together with a copy of the petition to USDA requesting approval for commercial release of the new varieties of tomato.

**Introduced Genetic Material**

Zeneca described the identity and function of the genetic material introduced into the tomato using the *Agrobacterium* transformation system (summarized on page 28 of summary document). Zeneca presented Southern blot analysis, PCR analysis, and segregation data to show that they have 1) properly identified the sequences that were inserted into the plant genome, 2) ascertained that the introduced genetic material was integrated at a single insertion site, and 3) showed that the inserts remain stably integrated through successive generations.

### **Identity and Function of Expression Products Encoded by the Inserted Genetic Material**

The only new protein that is expressed in the transgenic tomato is the enzyme aminoglycoside 3'-phosphotransferase II (APH(3')II), which is encoded by the *kan'* (*nptII*) gene originally isolated from transposon Tn5 isolated from *E. coli*. The *kan'* gene is used as a selectable marker. Zeneca stated that they carried out an open reading frame analysis of the entire inserted DNA and that, while the analysis showed the presence of several potential open reading frames, the only ones associated with plant DNA elements required for expression are in the opposite strand of the *kan'* gene and are therefore highly unlikely to be expressed as their expression would almost certainly interfere with APH(3')II expression. Such interference was not observed.

The insert also contains a fragment of the polygalacturonase (PG) gene which is responsible for the trait of the transgenic tomato. The PG gene fragment (which may be in the sense or antisense orientation) is driven by the Cauliflower Mosaic Virus 35S promoter. Transcription of the PG gene fragment results in the inhibition of the endogenous PG enzyme. PG enzyme is responsible for the normal breakdown of pectin molecules (a large polymer of galacturonic acid residues) in the cell wall of tomato fruits during ripening. Thus, the net result of the modification is that the pectin chains found in ripe transgenic tomatoes would be longer than those normally found in ripe tomatoes. According to Zeneca, this change increases the thickness of the tomatoes which are used to make processed tomato products and reduces waste due to spoilage.

### **Safety of the Introduced Protein**

As mentioned above, the new varieties contain only one added protein, namely APH(3')II. Zeneca noted that the safety of this protein in the development of new varieties of tomatoes has been addressed previously (21 CFR 173.170 and 21 CFR 573.130). Nonetheless, Zeneca presented data to show that the protein was expressed at extremely low levels in fresh tomato fruits and was essentially absent from processed products due to denaturation and inactivation (summarized on p. 17 of the summary document).

### **Compositional Analysis**

According to Zeneca, the only difference between the new and the parental variety of tomatoes is one of size distribution of pectin molecules. The agency has previously determined that this difference does not raise a safety concern. Not only is pectin widely consumed as a component of many fruits and vegetables, but it is a generally recognized as safe (GRAS) substance that is directly added to many food products as a gelling agent or as a

stabilizer (Summary of consultation with Calgene, Inc., regarding FLAVR SAVR™ tomatoes, May 17, 1994). In addition, Zeneca pointed out that pectin chain length breaks down during normal processing and that pectin chain length in processed products of the new tomato variety is well within the range commonly found in processed products of traditional tomatoes.

Zeneca stated that they tested for the glycoalkaloids chaconine and solanine but did not detect any. Zeneca also stated that data obtained from two laboratories indicate that the glycoalkaloid tomatine was detectable but at levels which were equivalent to levels in tomatoes obtained from the parental variety and in other traditionally bred tomatoes. We noted that, although we do not anticipate that tomatine levels in the transgenic tomatoes would be any different from levels in tomatoes obtained from the parental or in other traditionally bred tomatoes, tomatine levels reported in Zeneca's summary document were measured by an analytical laboratory using a non-standard analytical method. We routinely recommend that non-standard analytical methods be supported with satisfactory validation data. Because Zeneca stated that validation data were not included in the reports obtained from the analytical laboratory, we advised that Zeneca request such data and make sure that they are within the range generally accepted by analytical chemists. Zeneca stated that they intend to follow up on our suggestion to ensure that any data that they would rely upon have been properly validated.

Regarding nutrients, Zeneca stated that they have analyzed for vitamins A and C, as well for calorie content, fat, sodium, carbohydrate, fructose, glucose, dietary fiber, protein, calcium and iron and found no statistically significant differences between the new and traditional varieties in any of these parameters. Zeneca also noted that there were no differences in insoluble or soluble fiber content, in ash, titratable acidity, pH or Hunter color L:A:B: (a measure of color). These data are summarized on pp. 29-32 of the summary document.

## **Conclusions**

Zeneca has concluded, in essence, that the new tomato variety they have developed is not significantly altered within the meaning of 21 CFR 170.30(f)(2) when compared to tomato varieties with a history of safe use. At this time, based on Zeneca's description of its data and analysis, the agency considers Zeneca's consultation on this product to be complete.

Nega Beru, Ph.D.